

## 96-well Omega Bio-Tek DNA Prep

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This protocol uses the following purchased reagents:

Company	Kit/Item	Catalog Number
Omega Bio-tek	E-Z 96 Tissue DNA Kit	D1196-00
Fisher	Rayon Breathable Plate Covers	14-222-043

### Collecting Worms

1. Chunk a 1 cm square chunk from a recently starved plate of *C. elegans* to a 6 cm NGMA plate spotted with 100uL of overnight OP50 culture and dried overnight at 37°C.
2. Wait 2 days until the plate has plenty of L4 animals. Pick five L4's to two plates. Store plates at 20°C for four days. We want lots of gravid animals after four days, so be sure to watch their growth and move them to room temperature if needed.
3. Follow the procedure to bleach a 96-well plate of worms found in HighThroughputBroodSizePumpingAssay v2.0 protocol (steps 7-33 - through placing the Rayon-covered plates in a 20°C shaking incubator overnight).
  - a. For each 96-well plate of worms you want to prep, make 10-12 96-well growth plates that can be combined.
4. The following morning, make a 100 mg/mL solution of food from HB101 bacterial lysate (include Kanamycin at a final concentration of 50  $\mu$ M) in K Medium (recipe below). Using the electric repeat pipettor, add 5  $\mu$ L of food solution to all wells containing worms in the 96-well plates.
5. Incubate the plates in a humidity chamber at 20°C and 180 rpm until starvation.
  - a. Check the humidity chamber every other day to make sure there is still moisture.
  - b. It takes about a week for the majority of wells to starve
6. After the wells are starved, check each well. Mark the well if any of the following are an issue: mold contamination, bacterial contamination, empty, dehydration, or low numbers of worms. If you choose, record this information in a spreadsheet.
  - a. For mold contamination, we remove the largest mold puffs with a sterile pipette tip (making sure to change the pipette tip for each well).
  - b. For bacterial contamination, we simply remove all the contents from the well by aspiration and then wash the well three times with dH<sub>2</sub>O.

## DNA Prep

1. After the animals starve, prep DNA using the 96-well column kit from Omega Bio-tek.
  - Always conduct these preps in pairs as it is difficult to balance the combination of the column and collection plates in a centrifuge.
2. Combine all the animals from each plate that contains the same strains into the 96-well Round Well Plate of the kit.
3. Spin down the animals and remove as much supernatant as you can.
  - We use a 96-well aspirator (model # VP 177A-1) from V&P Scientific (see SetUp96WellAspirator protocol). About 150  $\mu$ l of liquid remained after aspiration.
  - **If you are prepping more than one plate at a time**, make sure to clean the 96-well aspirator between the plates:
    - Fill an old tip box with dH<sub>2</sub>O. Place under the aspirator to move the water through the tips.
    - Repeat the water wash.
    - Fill the tip box with 95% ethanol and move the ethanol through the tips.
    - Tip the entire manifold to the side with the vacuum attachment to make sure all liquid has been removed.
    - Run the aspirator for a few minutes after the ethanol rinse to make sure all the ethanol has evaporated.
    - Tip the aspirator to the side to remove all traces of ethanol.
4. Add 200  $\mu$ l TL Buffer and 25  $\mu$ l OB Protease Solution to each sample.
  - You can make a master mix for one plate by combining 21 ml of TL buffer with 2.625 ml of OB Protease Solution. Put the resulting mixture in a sterile trough and use a multi-channel pipette to add 225  $\mu$ l to each well.
  - Seal well with foil. Briefly vortex at 1200 rpm for 10 seconds to mix thoroughly.
5. Incubate at 60°C overnight or until your samples are lysed.
  - We found that just as with the DNAEasy kit from Qiagen, the samples were mostly lysed after 2 hrs. Some samples have embryos that remain in the lysate, but whether there are embryos doesn't seem to make a difference in the final outcome.
  - Note: The lysate should be clear and viscous after digestion is complete. Make sure the samples are completely lysed. Undigested material may clog the E-Z 96 DNA Plate in Step 11.
  - Shake or vortex the plate vigorously from side to side (do not shake up and down to avoid leaking around the caps). Hold the caps to ensure the plate is sealed properly. Ensure the lysate is completely homogeneous after shaking. If a gelatinous mass is visible, further digestion is required.
6. Add two volumes BL Buffer to each sample. A white precipitate may form at this step; it will not interfere with DNA isolation. Seal the plate with foil. Mix the sample by shaking or vortex the plate vigorously (side to side) for 1 minute.
  - Note: BL Buffer must be diluted with 100% ethanol before use.
  - Because of the 150  $\mu$ l in the well when we started the prep, two volumes of BL Buffer should be 650  $\mu$ l. However, when we performed this step we only added 450  $\mu$ l of BL solution, which is what you would need to add if there was no liquid in the well to begin with.

- For one plate, this can be added by adding 46 ml of BL Buffer to a sterile trough and adding 450  $\mu$ l to each well using a multi-channel pipette.
7. Briefly spin the plate at 2,500 rpm to collect any residue solution from the caps.
    - Note: Do not centrifuge for a prolonged time. Once the speed reaches 2,500 rpm stop the centrifuge.
  8. Place the E-Z 96 DNA Plate on top of a 96-well Square-well Plate (provided).
  9. Transfer all the lysate from Step 12 to each well of the E-Z 96 DNA Plate.
  10. Seal the E-Z 96 DNA Plate with AeraSeal film (provided).
  11. Centrifuge at 3,700 rpm for 10 minutes.
    - If the plate adaptors for your centrifuge rotor has a removable carrier, you might want to test if you can spin with the carrier. We find that we can not use the removable carrier when we spin.
    - When you spin, make sure to put the angled edge of the plates toward the center of the rotor. The square edge sticks out just enough to get caught on the inside of our rotor, which causes the plate to get stuck in a vertical position.
    - Note: Ensure that each sample has passed through the membrane in each well of the E-Z 96 DNA Plate. Longer centrifugation may be required if any lysate remains in any of the wells. If some lysate is still left in the wells even with increased centrifugation time, proceed to next step.
  12. Remove and discard the AeraSeal film.
  13. Add 500  $\mu$ L HBC Buffer to each well. Seal the plate with new AeraSeal film.
    - Note: HBC Buffer must be diluted with isopropanol before use.
    - For one plate, you can add 52.5 ml to a sterile trough and add 500  $\mu$ l using a multi-channel pipette.
  14. Centrifuge at 3,700 rpm for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate.
  15. Remove and discard the AeraSeal film.
  16. Add 600  $\mu$ L DNA Wash Buffer to each well. Seal the plate with new AeraSeal film.
    - Note: DNA Wash Buffer must be diluted with 100% ethanol before use.
    - For one plate, you can add 62 ml to a sterile trough and add 600  $\mu$ l using a multi-channel pipette.
  17. Centrifuge at 3,700 rpm for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate.
  18. Repeat Steps 21-23 for a second DNA Wash step.
    - \*Kruglyak lab does not do this\*
  19. Centrifuge at 3,700 rpm for 15 minutes. Discard the filtrate and the 96-well Square well Plate.

- Note: This step is critical for removing trace residual ethanol that might otherwise interfere with downstream applications. The plate can be further dried by placing the plates in an incubator or vacuum oven preset at 70°C to dry the membrane.

20. Transfer the E-Z 96 DNA Plate to a set of 96-well Racked Microtubes (provided).

21. Remove and discard the AeraSeal film.

22. Add 100  $\mu$ L Elution Buffer heated at 70°C to each well of the E-Z 96 DNA Plate. Seal the E-Z 96 DNA Plate with new AeraSeal film.

- For one plate, you can add 11 ml to a sterile trough and add 100  $\mu$ l using a multi-channel pipette.

23. Let sit at room temperature for 2-5 minutes.

24. Centrifuge at 3,700 rpm for 5 minutes.

25. Seal the 96-well Racked Microtubes with the Caps for Racked Microtubes and store the eluted DNA at -20°C.

- We prefer to seal the plates with foil seals.

K Medium Recipe (volumes in parentheses indicate the amount to add for a final volume of 500 ml)

51mM NaC (5.1 ml of 5M NaCl)

32 mM KCl (16 ml of 1M KCl)

3mM CaCl<sub>2</sub> (1.5 ml of 1M CaCl<sub>2</sub>)

3mM MgSO<sub>4</sub> (1.5 ml of 1M MgSO<sub>4</sub>)

- Mix the four salts with increasing water, then fill to 500 mL of dH<sub>2</sub>O.
- Filter sterilize with ThermoScientific Filter Unit (Cat #566-0020).
- Add 1.25 ug/mL filtered cholesterol (125 uL of 5 mg/mL cholesterol) (see recipe below).
- Mix, label, and store for up to two weeks. Be sure to check the K-medium prior to use for any floating particulate, which is a sign of contamination. If K-medium is contaminated, pour it down the drain and make new K-medium.

Cholesterol

- To make 10 ml, dissolve 50 mg of cholesterol in 100% ethanol.
- Filter the material using a 0.2  $\mu$ m filter (Cat. # SLLG025SS, Millipore)